

SUPERCRITICAL-FLUID EXTRUSION OF MILK PROTEIN CONCENTRATE
INTO PUFFED PRODUCTS

A Thesis

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ABSTRACT

Milk protein concentrate (MPC) is a high-protein, low-lactose dairy powder that contains caseins and whey proteins in the same ratio as milk. Although ideally suited for delivery of nutritionally superior products, MPC has not been extruded into commercial puffed snacks nor breakfast cereals due to its heat sensitivity. Meanwhile, apple pomace (AP) is a marginally utilized by-product of juice production and is also a good source of fiber and phenolic compounds. Its co-extrusion with MPC using low-temperature, low-shear supercritical-fluid extrusion (SCFX) technology to develop high-protein, shelf-stable products offers an economically and nutritionally attractive opportunity. In this study, SCFX was used to develop expanded products from MPC with added AP and sugar for direct consumption. The expansion ratios, density, porosity, water diffusion coefficient, color, mechanical properties and total phenolic content of the MPC extrudates containing three levels of AP (5%, 15% and 25%) and sugar (5%, 10% and 15%) were measured. The changes in texture, density and water activity of the MPC extrudates during storage were also evaluated. Two extruded commercial products were evaluated for comparison. The results showed that the MPC extrudates had low density, high porosity and good textural properties, which did not change during 9 months of storage. Adding AP at higher concentrations ($\geq 15\%$) adversely affected the expansion characteristics of the extrudates. At lower AP concentrations, the texture of the extrudates was comparable to commercial products. As expected, the addition of AP and sugar caused the product to appear darker in color. Additionally, SCFX processing increased the availability of phenolic

compounds in the extrudates, indicating improvements in the overall nutritional quality of the final product.

BIOGRAPHICAL SKETCH

Novita obtained a bachelor degree in Food Technology from Soegijapranata Catholic University, Semarang, Indonesia. During her undergraduate study, she was involved in a research project to evaluate the glucosinolates content of *Brassicca* vegetables after domestic processing. After graduation, she worked in the same university for one and a half year as lecturer before she decided to pursue a Master of Science degree at the Department of Food Science, Cornell University. Her master thesis research focused on the supercritical-fluid extrusion of milk protein concentrates and apple pomace.

*This thesis is dedicated to my family, Hari Gijanto, Liem Siok Nio, Leonardo, Leony
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CHAPTER 1

INTRODUCTION

1.1. Cooking Extrusion

Extrusion means a process to push a semi-solid material through a small opening (die). In food processing, extrusion can be classified based on its operating conditions into cold extrusion (for example in pasta making) and cooking extrusion (for example in puffed snack making). Cooking extrusion technology provides several functions such as conveying, mixing, cooking, kneading, shearing, and shaping (Fellow, 2000; Guy, 2001a).

Cooking extrusion is done in a very high barrel temperature, usually in the range of 100-180°C. The high temperature and high pressure allow a full transformation of raw materials in a short time (30-120 seconds) (Guy, 2001a). During cooking extrusion, raw materials are fed into the extruder barrel where the screws rotate to convey the food along the barrel. As materials move along the barrel, the screws knead them into semi-solid, plasticized dough with addition of moisture. Heat is generated due to the viscous dissipation during the extrusion, causing a rapid temperature rise. As the dough passes the die, pressure and shearing are further increased and as the dough is discharged, it expands to the final shape. The expansion during cooking extrusion happened due to rapid vaporization of the superheated moisture. After expansion, the rapid fall of temperature and the rise in viscosity, both due to moisture evaporation, rigidifies the cellular structure (Fellow, 2000).

1.2. Supercritical-Fluid Extrusion

Supercritical is the condition where a substance exists above its critical point and presents as liquid and gas. The critical point of CO₂ is 7.38 MPa and 31.1°C. Supercritical CO₂ (SC-CO₂) has high diffusivity and high dissolving power. It is also non-toxic and easily removed by depressurization (White et al., 2006).

Supercritical-fluid extrusion (SCFX) is an extrusion technology which involves the injection of supercritical-fluid, preferably carbon dioxide (CO₂), to expand the extrudates. The injection of supercritical fluid offers the possibility of low-temperature and low-shear puffing. Therefore, SCFX technology can overcome the limitations of conventional extrusion with regard to the process-related product characteristics (Rizvi et al., 1995). SCFX also minimizes the nutrient loss during extrusion of heat-sensitive materials.

Dissolved SC-CO₂ in the melt lower the pH during SCFX, which prevented Maillard reaction that causes further loss of essential amino acids. Moreover, SCFX extrudates have nonporous surface and homogeneous closed cell structure, which not only facilitate flavor encapsulation but also provide better textural control than steam-puffed extrudates (Cho & Rizvi, 2010). SCFX has been utilized to improve the functional properties of milk protein and to create puffed products from various heat sensitive materials such as whey protein, soy protein and fruit pomaces (Mustapha et al., 2012; Nor Afizah & Rizvi, 2014; Sharif et al., 2014; Sun et al., 2015).

The schematic diagram of supercritical fluid extruder is shown in Fig 1. Physical properties of SCFX extrudates can be manipulated through various process parameters, including die geometry, pressure-drop rate, residence time, ingredient composition and drying temperature (Cho & Rizvi, 2010).

1.3. Raw Material in Extrusion

Raw materials in cooking extrusion can be classified based on their functional roles. They are:

a. Structure-forming materials

Structure-forming materials contribute to the formation of cell walls; therefore, these materials need a certain degree of extensibility. Structure-forming materials are usually biopolymers with a certain optimum range of molecular weight. Starch polymers, such as wheat, maize, rice or potato, are very good at this function (Guy, 2001b). Protein may also be used to form structures although challenges has been reported in the extrusion of protein (Allen et al., 2007; Day and Swanson, 2013; Fernandez-Gutierrez et al., 2004; Onwulata et al., 2001).

b. Dispersed-phase filling materials

Microscopic examinations of extrudates reveal that several dispersed phases lie within the continuous starch structure in the extrudates. The most common dispersed materials are protein and fibrous materials such as cellulose or bran. The size and shape of dispersed materials depend on their original particle size and their resistance to shearing (Guy, 2001b).

The presence of dispersed-phase materials will not only reduce the elasticity of raw material but also disrupt the cell walls causing less potential for expansion. Addition

of fiber-rich ingredients, such as fruit pomace, fruit pulp, and dehydrated vegetables, significantly reduces the expansion ratio of the extrudates (Bisharat et al., 2013; Karkle et al., 2012; O'Shea et al., 2013).

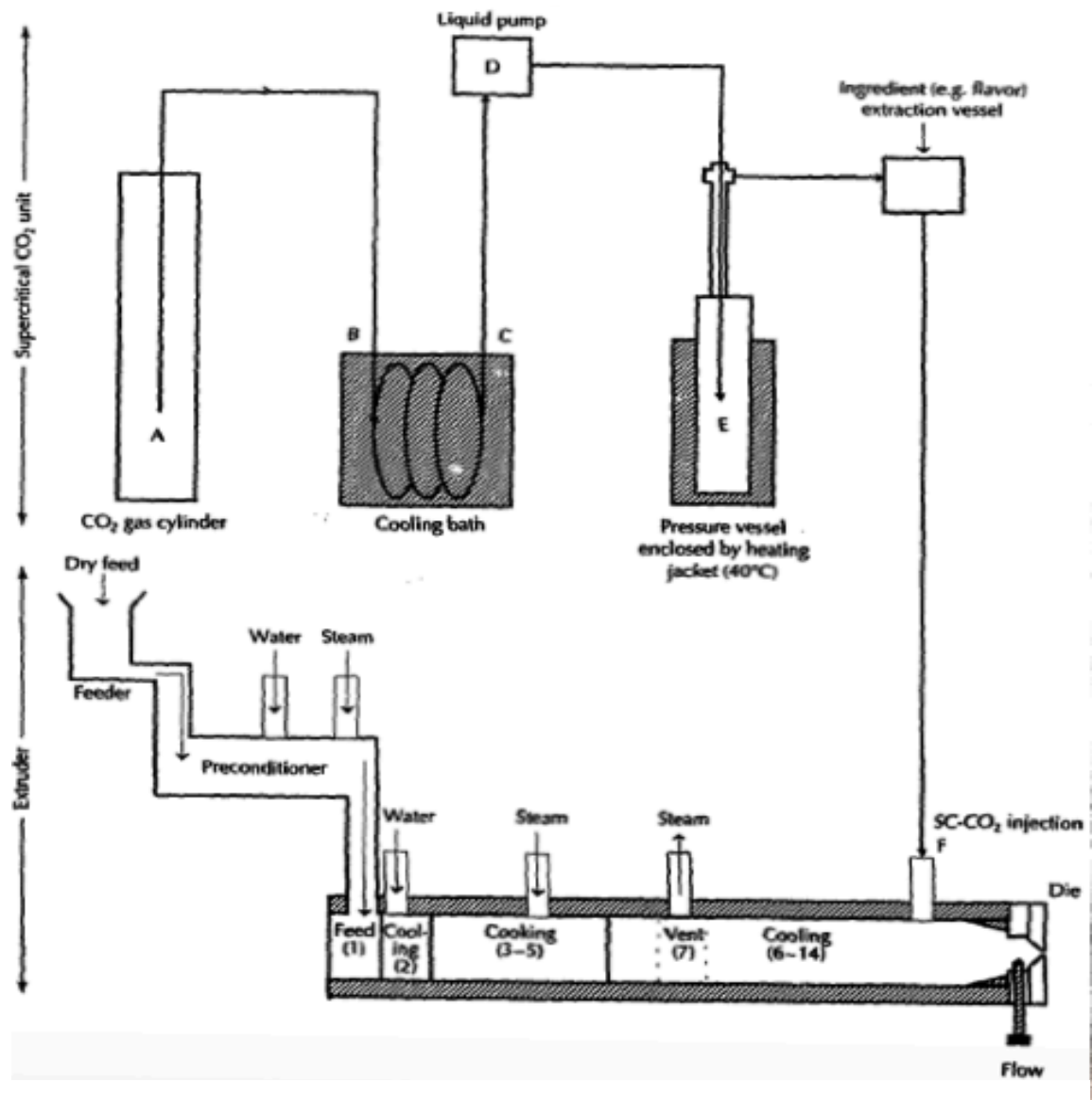


Fig 1. Supercritical Fluid Extrusion Principle (Rizvi et al., 1995)

c. Plasticizers and lubricants

Water during extrusion serves to reduce the dissipation of mechanical energy by plasticizing the dry polymer forms, transforming them from solids to deformable plastic semi solids (Guy, 2001b). Addition of moisture reduces the viscosity of the dough, therefore reduces the friction between the dough and screw. Moisture also reduces the glass-transition temperature (T_g) and the flow temperature (T_f), which represents the temperature where the blend is plasticized enough to flow through capillary. The reduction of T_g and T_f affect the starch gelatinization greatly and as a result, changes in the microstructure are observed. Increased moisture leads to reduction of expansion ratio and increase in density. Thus, the amount of water during extrusion needs to be controlled closely (Bisharat et al., 2013; Karkle, et al., 2012; Zarzycki et al., 2015).

1.4. Expansion Mechanism in Extrusion

The proposed expansion mechanism in extrusion includes the following 5 major steps: order-disorder transformations, nucleation, extrudate swell, bubble growth, and bubble collapse. First, the high shear, pressure, and temperature inside the extruder allow the transformation of the structure forming materials into viscoelastic melts. Nucleation of bubbles within the polymer melt occurs during extrusion, both at sites where small air bubbles or dispersed particles were entrapped during the extrusion process. These bubbles grow as the melt leaves the extruder die due to a moisture flash-off process, when the high pressure of the superheated steam generated by moisture vaporization at nuclei overcomes the mechanical resistance of the viscoelastic melt. The bubble growth ceases upon cooling, when the viscoelastic matrix becomes glassy and no longer allows expansion to take place (Moraru & Kokini, 2003).

The expansion mechanism during SCFX is similar to the expansion in cooking extrusion. The main difference is the bubble growth that happens due to the volume expansion from the depressurization of SC-CO₂ and all the expansion process happened in temperature lower than 100°C (Rizvi et al., 1995).

There are several factors that affected expansion during extrusion, as reviewed by (Moraru & Kokini, 2003). The raw materials have great impacts on the viscoelasticity of the melt. The rheological properties of the polymeric matrix have the leading role in expansion, since they determine the resistance of the cell wall during bubble growth. Process parameter during extrusion that may change the rheology of the melt, such as moisture content, screw speed, extrusion temperature and mechanical energy input, has shown strong correlation to the expansion of extrudates.

1.5. Milk Protein Concentrate

Milk protein concentrate (MPC) is a complete dairy protein complex that contains both caseins and whey proteins in the same ratio as milk. The manufacturing of MPC involves the concentration of pasteurized skim milk, mostly by ultrafiltration and diafiltration that separates most of the lactose and minerals, followed by water removal usually by spray drying or evaporation. The protein content in MPC depends on the degree of ultrafiltration and diafiltration employed and it categorizes the MPC into low-protein powder (<40 wt.% protein), medium-protein powder (60-70 wt.% protein) and high-protein powder (>80%) (Sikand et al., 2011; Uluko et al., 2015).

MPC usage as ingredients is increasing because of its high protein, low lactose content and excellent functional properties, such as foaming, emulsifying, viscosity, gelling and heat stability (Agarwal et al., 2015; Uluko et al., 2015). Some examples of MPC applications in food products are shown in Table 1.

Table 1. Applications of MPC

Products	Functions	References
Cheese	Increased yield	Caro et al., 2011; Shakeel-Ur-Rehman et al., 2003
Ice cream	Increased nutritional value (high protein and low lactose)	Patel et al., 2006
Yoghurt	Increased protein content, viscosity	Guzmán-González et al., 1999
High-protein nutrient bar	Improved stability during storage	Banach et al., 2014

MPC must be completely dissolved to fully express its functional properties, thus manufacturers regard the solubility of MPC as a critical property. The solubility of MPC decreases when its protein content increases (Crowley et al., 2015), therefore MPC with protein content higher than 80% have a very low solubility that impairs the functional properties. The rate-limiting step during the rehydration of MPC was the dissolution of inter-linked casein micelles (Mimouni et al., 2009). Therefore, the solubility of the MPC is greatly affected by the particle's structure and composition, which is defined by the process condition during its manufacture and applications. Various processes have been proven to alter the structure and composition of the caseins in MPC such as heat treatments (Banach et al., 2013; Uluko et al., 2015), high-pressure treatments (Uluko et al., 2015) and extrusion porosification (Bouvier et al., 2013). Heat treatments, such as spray drying, toasting and cooking extrusion, promote micelle-micelle interaction between caseins that build up the cross-link between casein

micelles through both covalent and non-covalent (hydrophobic) interactions (Banach et al., 2013; Bouvier et al., 2013; Uluko et al., 2015). On the other hand, the injection of CO₂ gas to MPC powder during extrusion porosification improves the solubility of MPC by promoting coarse network formation instead of closely knitted cross-links between casein micelles (Bouvier et al., 2013).

1.6. Apple Pomace

Apple pomace (AP) is the primary by-product of the apple juice industry that poses environmental problems due to the large amounts produced every year all around the world (Lu & Yeap Foo, 2000; Reis et al., 2014). Many researches have been attempted to utilize AP as value-added products such as bio-fuels, fertilizer or pectin production. However, the very large volumes produced each year exceed existing usage and new applications for AP are required (Hwang et al., 1998; Reis et al., 2014).

AP is composed of mainly the insoluble components of apple, such as cellulose, hemicellulose and pectins, which are not digested by enzymes in the human body and thus classified as dietary fibers (Hwang et al., 1998). Polyphenols are still abundant in the AP, which are responsible for the antioxidant activity of the AP. Polyphenolic compounds present in the AP include phloridzin, 3-hydroxyphloridzin, chlorogenic acid, epicatechin and quercetin glycosides (Lu & Yeap Foo, 2000). Thus, AP is a cheap and readily available source of dietary fiber and antioxidant that needs to be commercially exploited.

AP has been incorporated into the development of some new functional food, such as bakery products and extrudates. As expected, AP increased the total dietary fiber content in extruded snacks and bakery products. However, processing can cause the loss of bioactive compounds, especially ones that involve heat. The loss of phenolic content can be attributed to the thermal degradation of either free or conjugated bioactive compounds that may be polymerized further through chemical reactions with Maillard by-products, resulting in the formation of complexes. Despite the degradation of the bioactive compounds, heat treatment increases the antioxidant activity. This is probably due to the high scavenging activity of the Maillard reaction products (Leyva-Corral et al., 2016; Reis et al., 2014).

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CHAPTER 2

SUPERCRITICAL-FLUID EXTRUSION FOR MILK PROTEIN CONCENTRATE INTO PUFFED PRODUCTS

2.1. Introduction

Milk protein concentrate (MPC) is a high-protein dairy powder that contains both caseins and whey proteins in their native states and in the same ratio as in milk. Whey proteins in MPC are mostly in their undenatured state and caseins maintain their micellar structure with no significant change in size and shape after manufacturing (Martin et al., 2010). MPC is an ideal ingredient for wide applications in the food industry due to its highly nutritional profile and beneficial functional qualities such as binding water, imparting heat stability, and forming gels, foams and emulsions (Agarwal et al., 2015). Milk proteins are also capable of binding bioactive compounds and thereby promoting their delivery to the body (Livney, 2010).

Some current and suggested uses of MPC include its incorporation in cheese making (Shakeel-Ur-Rehman et al., 2003), ice cream making (Patel et al., 2006), and modifying high-protein bar formulations (Banach et al., 2014) to improve nutritional and sensory qualities. Applications of MPC into any food products are greatly affected by its solubility and functionality. Many recent studies have focused on processes to manipulate MPC performance, including spray-drying, extrusion porosification (Bouvier et al., 2013), toasting and cooking extrusion (Banach et al., 2013). High-temperature extrusion-induced disulfide bond formation reduces the solubility and

water holding capacity of the MPC resulting in puffed products with low expansion ratios and poor textural qualities (Allen et al., 2007; Day and Swanson, 2013; Fernandez-Gutierrez et al., 2004; Onwulata et al., 2001). The effects of cooking extrusion on the protein structure become more pronounced as the temperature and shear force are increased (Banach et al., 2013). Therefore, MPC has not been commercially manufactured into puffed snacks or breakfast cereals. However, the addition of ground, extruded MPC with 80 percent protein was reported to enhance the texture and other sensory attributes of high-protein nutrition bars when compared with bars made with unmodified MPC (Banach et al., 2014).

Apple pomace (AP) is a by-product from juice processing industries, which is non-optimally utilized and poses a waste disposal problem. It is rich in fiber and phenolic compounds, which makes it a good source of highly desirable nutrients (Lu and Yeap Foo, 2000; Figuerola et al., 2005). Incorporation of AP into MPC extrudates complements and enhances the nutritional quality of the extrudates; however, thermal degradation of the phenolic compounds in AP has been observed during cooking extrusion (Leyva-Corral et al., 2016; Reis et al., 2014). Adding AP has also been reported to impart adverse effects on the radial expansion ratio and mechanical properties of the extrudates (Karkle et al., 2012). Including fiber-rich ingredients in extruded products reduces the elastic modulus and increases the hardness of the extrudates (Karkle et al., 2012; Robin et al., 2011) and therefore poses a challenge in making fiber-rich puffed products.

Sugar is also often added to improve the sensory quality of food products. However, sugar is known to adversely affect the expansion, density, and texture of extrudates (Pitts et al., 2014; Fan et al., 1996; Barrett et al., 1995). Sugar also promotes Maillard browning in protein-enriched extrudates made with conventional high-temperature cooking extrusion; Maillard browning results in loss of some essential amino acids, like lysine (Banach et al., 2014; Singh et al., 2007). Commercialization of MPC-based extruded products containing AP relies on addressing these quality issues and generating consumer-acceptable products.

Supercritical fluid extrusion (SCFX) is an alternative extrusion technology that uses a supercritical fluid, preferably carbon dioxide, as the blowing agent for making puffed products. It offers the possibility of low-temperature puffing, thereby minimizing the adverse effects of high temperature on heat-sensitive ingredients (Rizvi et al., 1995). SCFX had been used to create MPC-starch-pomace extrudates with good expansion characteristics, low densities, and good texture profiles (Sun et al., 2015). However, puffed extrudates solely from MPC without starch have not been developed and their storage characteristics have also not been studied. Furthermore, the effects of adding AP and sugar on the characteristics of MPC-based, SCFX extrudates remain to be quantified.

The objectives of this study were to quantify the physicochemical properties of MPC extrudates produced by SCFX and to evaluate their textural changes during storage. The effects of added AP (5%, 15% and 25%) and sugar (5%, 10% and 15%) on the

extrudates characteristics were also evaluated.

2.2. Materials and Methods

Materials

MPC-80 powder was provided by Glanbia Nutritionals (Sioux Falls, SD, USA). AP was acquired from the apple processing facility at Cornell Orchard (Ithaca, NY, USA) and subsequently oven-dried (80°C) until the moisture content reached 5%wb and then milled into powder. The composition of the MPC-80 and AP are shown in Table 2. Lecithin, distilled monoglycerides (Dimodan) and sugar were purchased from ADM Company (Decatur, IL, USA), Danisco (Kansas City, MO, USA) and Walmart (Ithaca, NY, USA), respectively.

Table 2. Typical compositions of MPC-80 and AP (g/100g, wet basis)

	MPC-80	AP
Protein	81.56	2.40-6.40
Ash	6.79	0.50-1.88
Fat	1.74	1.57-5.4
Moisture	5.26	1.2-11
Other milk solid (lactose, NPN, etc)	4.65	-
Total dietary fiber	-	60.7-89.80
Soluble		4.14-20.00
Insoluble		56.5-81.6

Source : MPC – Glanbia Nutritionals product specification; AP – Figuerola et.al., 2005; Hwang et.al., 1998; and Karkle et.al., 2012

Blend Preparation

Four dry feed formulations were prepared according to the compositions shown in Table 3. The dry powders were mixed for 16 minutes (8 minutes clockwise and 8 minutes counter-clockwise) using a Day (Cincinnati, OH, USA) horizontal single ribbon blender. Mixed formulation powders were kept in sealed buckets until the

extrusion process the next day. Formulation F3 was also extruded with three levels (5 wt.%, 10 wt.% and 15 wt.%) of added sugar.

Table 3. Extrusion formulations (g/100g, wet basis)

Ingredients	F1	F2	F3	F4
MPC-80	98	93	83	73
Apple pomace	0	5	15	25
Lecithin	1	1	1	1
Dimodan	1	1	1	1

Extrusion Processing

All formulations were extruded in a co-rotating twin-screw extruder (Wenger TX-52 Magnum, Sabetha KS, USA). Sugar was added into the water used in the extrusion. The extruder had a Length/Diameter ratio of 28.5:1 and was equipped with four supercritical carbon-dioxide (SC-CO₂) manifolded injection valves positioned at the L/D ratio of 24. Two circular dies (3.8 mm dia) were used. The following parameters were held constant during extrusion: feed rate (35 kg/hr); pressure (1800 psi/12,410 kPa); water input (14.0 - 14.7 kg/hr.); SC-CO₂ injection rate (0.7 – 0.75 kg/hr) and screws speed (80 rpm). The specific mechanical energy (SME) and the temperature of the dough exiting the die (die temperature) were recorded for each formulation. The extrusion processing was done twice for each formulation.

Sample Collection and Handling

Extrudates were cut using automatic rotating knives at the die exit into ~ 0.5 cm and ~5 cm long cylindrical samples, dried at 80°C for 90 minutes and sealed in plastic

bags until further analysis. Two commercial puffed breakfast cereals (Kix and Capt'n Crunch Berry) obtained from the local market were also evaluated for comparison.

Expansion Ratios

Expansion ratios of the samples were measured according to the method of Alvarez-Martinez et al. (1988). The specific length of the samples was determined as the ratio of their respective lengths and masses. Radial expansion ratio was calculated using Eq. 1 below:

$$\text{Radial Expansion Ratio (RER)} = \frac{D_e^2}{D_d^2} \quad \text{Eq. 1}$$

where, D_e is the diameter of the extrudates and D_d is the diameter of the die.

Longitudinal expansion ratio is calculated as the ratio of melt velocity and extrudates velocity. This ratio can be expressed in different terms derived from the mass balance around extruder by Alvarez-Martinez et al. (1988) shown in Eq. 2.

$$\text{Longitudinal Expansion Ratio (LER)} = \left(\frac{L_e}{L_d} \right) \left(\frac{1-M_d}{1-M_e} \right) \quad \text{Eq. 2}$$

where L_e is the specific length of the extrudates (m/g); L_d is the specific length of the unexpanded melt coming out of the die (m/g); M_d is the total moisture in the unexpanded melt (w/w, wb) and M_e is the total moisture in the extrudates before drying (w/w, wb).

Density and Porosity

Piece density was calculated using Eq. 3 :

$$\text{Piece density (g/cm}^3\text{)} = \frac{4m_e}{\pi D_e^2 l_e} \quad \text{Eq. 3}$$

where, m_e is the mass (g), D_e is the average diameter (cm) and l_e is the length of the dried extrudate sample (cm) (Alam et al., 2014; Karkle et al., 2012).

Solid density measurements were done using the method from O'Shea et al. (2013) with modifications. The extrudates were milled and sieved through a 60-mesh sieve. A 15 ml graduated cylinder was filled with the extrudates powder and then tapped gently until there was no further reduction of volume. Mass of the powder was then recorded and the ground powder was kept for color analysis. Solid density was calculated as the ratio of mass to volume. Porosity was calculated using Eq. 4. Measurements of piece density, solid density, and porosity were replicated five times for each set of extruded products.

$$porosity = \frac{solid\ density - piece\ density}{solid\ density} \quad Eq. 4$$

Color

Un-extruded mixed powder and the ground extrudate samples were analyzed to determine the color change during extrusion using a Konica Minolta's Chroma Meter CR400. Five replicate measurements of L^* , a^* , and b^* values were collected for each product. The color change resulting from the SCFX process was expressed as ΔE (Ondo et al., 2013).

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad Eq. 5$$

Rehydration Test

Representative extrudate samples of known mass were immersed in 50g of deionized water per gram of sample at 20°C. The rehydrated samples were taken out of the water and weighed once every 30 seconds for the first 5 minutes and then once every minute after that until a constant weight was reached. The samples were then spread on a sieve for three minutes to drain excess water and the weight gain compared to the initial mass was recorded (Bisharat et al., 2013; Cunningham et al., 2008).

Water diffusion coefficient was calculated using Fick's equation for a cylindrical geometry as follows:

$$X_t = X_e + (X_0 - X_e) \frac{4}{\beta_n^2} \exp\left(\frac{-\beta_n^2 D_{eff} t}{R_0^2}\right) \quad \text{Eq. 6}$$

where X_t is the moisture content of the sample at time t (w/w, db); X_e is the moisture content at equilibrium (w/w, db); X_0 is the initial moisture content (w/w, db); β_n is the first root of Bessel function ($\beta_1 = 2.4048$); D_{eff} is the effective diffusion coefficient (m^2/s); t is the rehydration time (s); and R_0 is the initial radius of extrudates sample (m). A plot of $\ln(X_t - X_e)/(X_0 - X_e)$ versus t yields a straight line with gradient $\frac{-\beta_n^2 D_{eff} t}{R_0^2}$ from which the values of D_{eff} may be calculated (Cunningham et al., 2008).

Mechanical Properties

Prior to mechanical properties analysis, extrudates were conditioned to four different water activities at room temperature ($\sim 22^\circ\text{C}$) by placing them in desiccators with four different saturated salt solutions: LiCl (a_w 0.113), CH_3COOK (a_w 0.225), K_2CO_3 (a_w 0.432), and NaBr (a_w 0.591).

Stress-strain curves of the samples were obtained using a TA-XTplus equipped with a 50 kg load cell and a cylindrical probe (35mm dia.). One layer of extrudates was arranged in a cylindrical container of 48 mm diameter. The compression test was done on the extrudates to 70% strain at the speed of 1 mm/s and at trigger force of 10 g.

Hardness, yield stress and crispness of the sample were determined from the stress-strain curves. Hardness was calculated as the maximum force; yield stress was the stress value when the sample began to fracture or deform; crispness was indicated by the normalized number of peaks (Np), which was calculated using Eq.7 (Dogan and Kokini, 2007).

$$Np = \frac{\text{total number of peaks}}{\text{distance traveled by the probe}} \quad \text{Eq. 7}$$

Modeling of Compressive Stress-Strain Behavior for Cellular Solid

The initial section of the stress-strain curves up to the yield point was observed to be curvilinear, as is often the case with porous structures. To characterize the curvature, the initial section was described by a power law-like equation as follows:

$$\sigma = M\varepsilon^n \quad \text{Eq. 8}$$

where σ is the stress (kPa) and ε is the strain. M and n are estimated constants, where n describes the curvature of the graph and M is the elastic coefficient, which reduces to the modulus of elasticity when n equals 1.

Total Phenolic Content

Total phenolic contents of formulations F2-F4 before and after extrusion were determined with the methods in Zhu et al. (2015). Free phenolic compounds were extracted from milled samples using acetone (80%). Two grams of sample was mixed for 5 min into 50 ml of acetone, and then centrifuged at 3000 rpm for 10 minutes. The supernatants were collected and were subsequently dried in a rotary evaporator at 45°C. The dried extract then was then reconstituted into 10 ml de-ionized water. The bound phenolics were extracted from the residues of the free phenolic extraction by digestion using 20 mL of 2 M NaOH. The mixtures were then neutralized with HCl (pH 2.0). Hexane extraction removed any lipids present. Bound phenolics were extracted from the remaining solution using 100 ml ethyl acetate. The ethyl acetate fractions were evaporated at 45°C and then reconstituted into 10 ml de-ionized water. All extracts were stored at -36°C until further analysis.

Total phenolic content of samples was determined by the Folin-Ciocalteu colorimetric method. Sample extracts and gallic acid standard were diluted using de-ionized water. The Folin–Ciocalteu reagent was added and then neutralized using 7% sodium carbonate solution. After 90 minutes, the absorbance of the mixture was measured at 760 nm using a UV-visible spectrophotometer, Genesys 20 (ThermoScientific, Waltham, USA). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g dry weight.

Extrudate Properties during Storage

MPC extrudates (F1) were sealed in plastic bags and stored for 9 months at room

temperature to quantify the effects of storage on the extrudates. The hardness, crispness, density, and water activity of the extrudates were periodically measured during storage.

Statistical Analysis

Statistically significant differences between the results were analyzed with a one-way ANOVA at 95% confidence level with R. Post hoc comparison was made based on p-value (0.05) with a Tukey HSD test.

2.3. Results and Discussions

Physical characteristics of MPC extrudates

Previous studies reported challenges with milk protein extrusion wherein disulfide bond formation and protein aggregation reduced the elasticity of the dough, consequently reducing extrudate expansion hampering their physical qualities (Allen et al., 2007; Day and Swanson, 2013; Fernandez-Gutierrez et al., 2004; Onwulata et al., 2001). In this study, SCFX was effectively utilized to extrude 98% MPC-80 powder into puffed products of lower density, higher porosity, lighter color and better textural properties (Table 4) than those reported in previous studies (Allen et al., 2007; Fernandez-Gutierrez et al., 2004) and the evaluated commercial products.

The low-temperature and low-shear during SCFX minimizes the effects of extrusion on the proteins, thus prevents the reduction of the proteins solubility and water holding capacity (Banach et al., 2013; Nor Afizah and Rizvi, 2014). Consequently, the dough

elasticity, and therefore its gas-holding capacity, was preserved and products with high expansion ratios and porosities were obtained.

From the mechanical properties analysis, typical jagged stress-strain responses of porous materials were also observed in the MPC extrudates (Fig 2). As a result of higher porosity, the MPC extrudates were not as hard as the two commercial products, which indicates less resistance during compression and biting. The MPC extrudates were also crispier than Kix and almost as crispy as Crunch Berry (Table 4). This showed that the MPC extrudate's texture could be tailored to simulate commercial puffed products.

SCFX also imparted minimum Maillard browning to MPC extrudates, which is a critical issue with conventional extrusion of milk protein that was reported in previous studies (Day and Swanson, 2013; Fernandez-Gutierrez et al., 2004). The SCFX samples had a light yellow color as shown in Fig 3, with a high L^* value and low a^* and b^* values (Table 4). The small ΔE value of the MPC extrudates indicates minimum color changes of the MPC after SCFX. In addition to the low-temperature and low-shear, the acidic condition generated during SCFX due to the formation of carbonic acid in the dough from SC-CO₂ injection also limits Maillard browning. Low pH decreases the number of reactive unprotonated amino groups and the rate of lactosylation which has been reported to minimize the Maillard reaction (Casal et al., 2006).

Table 4. Physical characteristics of MPC extrudates and commercial products

Characteristics	MPC extrudates	Kix	Crunch Berry
Radial expansion ratio	11.5±0.78	-	-
Piece density (g/cm ³)	0.12±0.005	0.15±0.005	0.18±0.006
Porosity	0.80±0.012	0.66±0.007	0.73±0.030
Hardness (N)	69.02±4.08	407.22±1.299	129.198±3.709
Crispness	9.14±0.28	2.75±0.266	10.06±0.135
Yield stress (kPa)	3.14±0.73	5.62±0.759	6.13±0.451
D _{eff} (m ² /s)	7.92x10 ⁻⁰⁸ ±2.51x10 ⁻¹⁰	-	-
Color		-	-
L*	90.03±0.36		
a*	-2.81±0.06		
b*	17.15±0.27		
ΔE	9.20±0.263		

Values are expressed in mean ± standard error

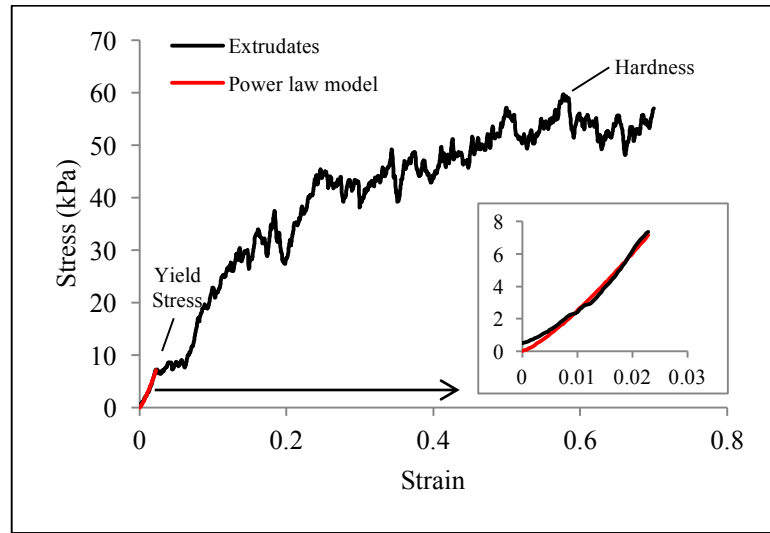


Fig 2. Stress-strain curve of MPC extrudates

MPC extrudates characteristic during storage

There was no significant change in the appearance (Fig 3) and the texture (Fig 4a) of MPC extrudates during 9 months of storage in sealed plastic bags at room temperature. On the other hand, the density and water activity of MPC extrudates slightly increased during the storage (Fig 4b). Textural quality degradations during the

MPC extrudates storage were impeded due to both the MPC's high glass transition temperature and the low moisture absorption rate. The glass transition temperature (T_g) of MPC-80 at a_w 0.47 is 353 K (Pugliese et al., 2016), meanwhile the T_g of corn starch at approximately the same moisture level is 334.4 K (Liu et al., 2009). This implies that MPC extrudates are more stable during storage compared to corn starch extrudates. High T_g delays the transition from crispy glassy amorphous polymer into its rubbery state. MPC is also known for its slow rehydration properties (Bouvier et al., 2013) which is favorable during storage since moisture acts as plasticizer and reduces the T_g of the MPC extrudates.



Fig 3. Photograph of MPC Extrudates during storage

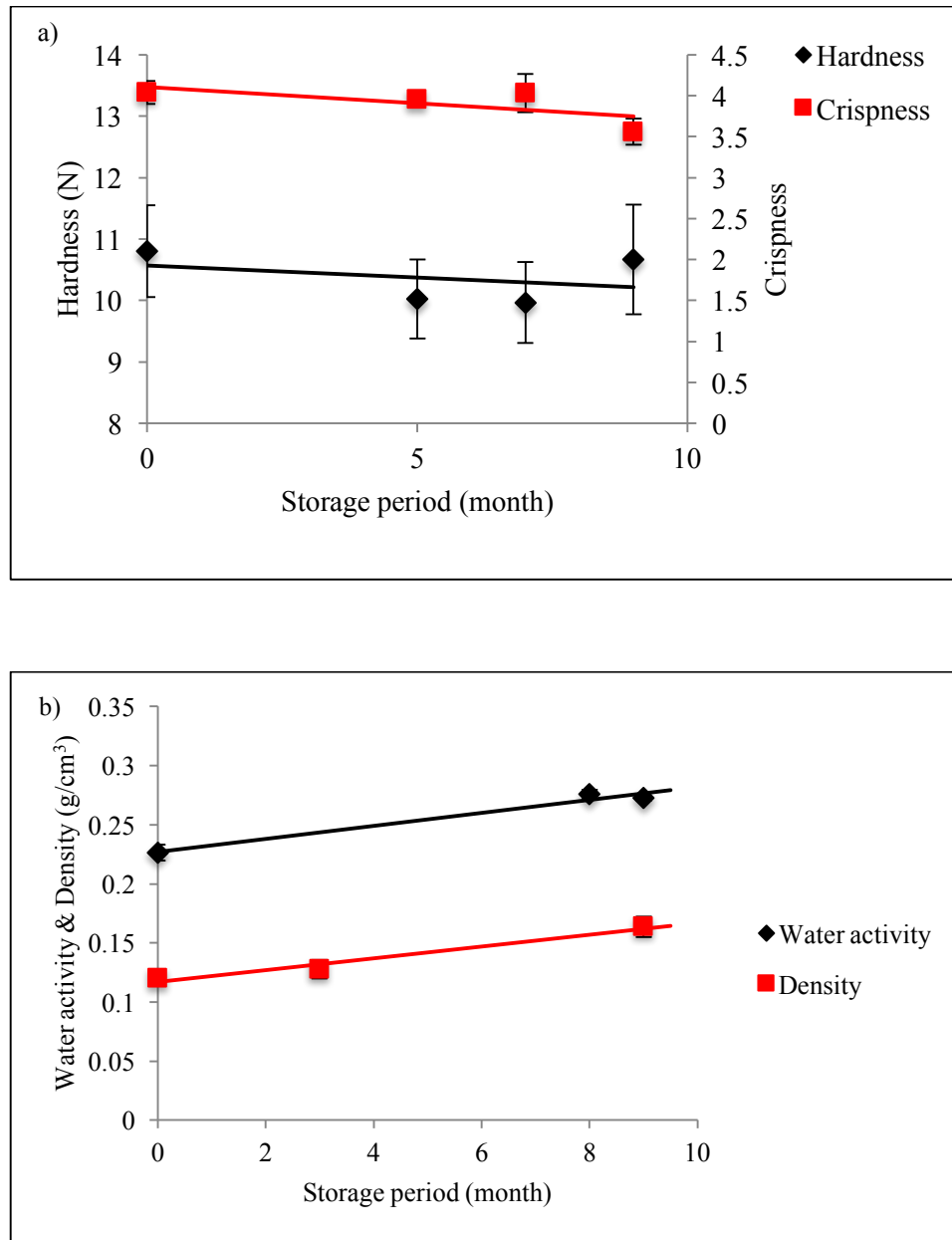


Fig 4. Changes of MPC extrudates properties during storage: a) texture and b) density and water activity. Points are mean value with standard error bar

Effects of AP addition

Similar to the results of previous studies where fiber-rich materials hindered radial expansion during extrusion (Karkle et al., 2012; O'Shea et al., 2013; Pai, Blake,

Hamaker, & Campanella, 2009), adding AP also decreased the radial expansion ratio (RER) of the MPC extrudates (Fig 5). The RER decreased linearly as the AP concentrations were increased, which suggested a reduction of about 0.18 RER per 1 wt% added AP. The RER decreased due to the insoluble dietary fiber, which increased the melt viscosity and decreased the melt elasticity, thus affecting its ability to expand. The increase in the viscous properties of the melt due to AP was demonstrated by the increasing SME during extrusion as the AP concentration was increased (Table 5).

In the melt, the insoluble fiber from AP tends to exist as filler in the protein matrix and to align parallel to the flow direction of the melt (Karkle et al., 2012; Robin et al., 2011). The fiber reinforces the protein matrix and thus strengthens the melt with the same mechanism as fiber reinforcement in composite polymers (Callister and Rethwisch, 2008). Consequently, more energy is required from the SC-CO₂ to expand the melt, which also explains the lower RER correlated with higher AP concentrations in MPC extrudates.

However, the negative effects of AP on the RER extrudates were less pronounced in extrudates made by SCFX than in extrudates made by conventional cooking extrusion (Karkle et al., 2012). Also, contrary to the previous studies (Karkle et al., 2012; Pai et al., 2009), decreased radial expansion in the MPC extrudates was not compensated by an increased longitudinal expansion ratio (LER) (Fig 5). In cooking extrusion of fiber-rich materials, fiber restrains the radial expansion of the extrudate, thus excessive energy from the vaporization of the superheated steam is used to expand

longitudinally. It is difficult to optimize the energy in steam-driven expansion because the moisture acts as both blowing agent and plasticizer. On the other hand, the expansion during SCFX can be easily optimized by adjusting the SC-CO₂ injection rate (Rizvi et al., 1995), thus radial expansion can be maximized without excessive steam energy causing longitudinal expansion.

The effects of added AP on density and porosity were in line with the RER, where a significant increase in piece density and a corresponding decrease in porosity were only observed in the MPC extrudates with high AP concentrations (15-25 wt.%). However, even though AP caused significant reductions in radial expansion, MPC extrudates with 0-15 wt.% AP still have comparable density and porosity to the commercial products (Table 6).

As expected, the extrudates with higher AP concentrations and lower porosities exhibited significantly lower water diffusion coefficients (Table 7) similar to the water diffusion characteristics reported for other porous material (Bisharat et al., 2013; Bouvier et al., 2013). The small diffusion coefficient is desirable since delayed moisture absorption will help maintain the texture and prolong the bowl life of the MPC extrudates if consumed as breakfast cereal. MPC extrudates with AP were able to maintain their shape after 5 minutes of submersion in DI water (Fig 6).

Higher AP concentrations magnified the color change (ΔE) resulting from SCFX. The extent of browning increased along with the concentration of AP (Fig 7, Table 8). As

more AP was added, more sugar became available to react with the MPC in Maillard browning. Even though the acidic environment in the barrel during SCFX hindered Maillard browning, it was not eliminated. Moreover, added AP also caused higher extrusion temperature at the die (Table 5) due to more viscous dissipation of mechanical energy into heat, which might contribute to Maillard browning despite the SC-CO₂-induced acidic environment in the extruder barrel.

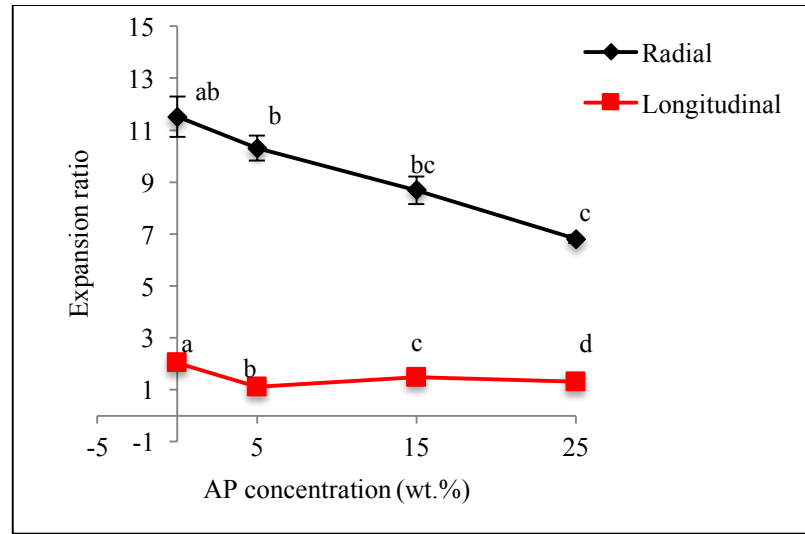


Fig 5. Expansion characteristics of MPC extrudates and the effects of added AP. Points are mean value (n=10) with standard error bar. Different letters indicate significant difference (p<0.05)

AP concentration (wt.%)	SME (Whr/kg)	Die temperature (°C)
0	35.45	73
5	35.45	77
15	40.10	78
25	41.65	87
Sugar concentration (wt.%)		
5	43.2	86.6
10	46.3	90.5
15	46.3	98

Table 6. Density and porosity of extrudates samples

AP concentration (wt.%)	Piece density (g/cm ³)	Solid density (g/cm ³)	Porosity
0	0.12±0.005 ^a	0.694±0.021 ^a	0.80±0.012 ^a
5	0.12±0.003 ^a	0.703±0.028 ^a	0.82±0.008 ^a
15	0.17±0.011 ^b	0.753±0.006 ^a	0.75±0.022 ^b
25	0.21±0.006 ^c	0.724±0.032 ^a	0.71±0.009 ^c
<i>Commercial product:</i>			
Kix	0.15±0.005	0.44±0.020	0.66±0.007
Berry Crunch	0.18±0.006	0.68±0.040	0.73±0.030

Values are expressed in mean ± standard error (n=10). Values with different letters are significantly different (p<0.05).

Table 7. Diffusion coefficient of MPC extrudates with added AP

AP concentration (wt%)	D _{eff} (m ² /s)
0	7.92x10 ⁻⁰⁸ ±2.51x10 ^{-10a}
5	5.160x10 ⁻⁰⁸ ±8.88x10 ^{-09a}
15	2.604x10 ⁻⁰⁸ ±7.74x10 ^{-09b}
25	0.592x10 ⁻⁰⁸ ±1.54x10 ^{-10c}

Values are expressed in mean± standard error. Different letters indicates significant difference (p<0.05)

Table 8. L*-a*-b* color values of MPC extrudates with different AP concentration

AP concentration (wt.%)	L*	a*	b*	ΔE
0	90.03±0.36 ^a	-2.81±0.06 ^a	17.15±0.27 ^a	9.20±0.263 ^a
5	82.47±0.34 ^b	1.21±0.24 ^b	18.98±0.33 ^b	12.95±0.498 ^b
15	75.01±0.23 ^c	3.44±0.04 ^c	18.47±0.41 ^{ab}	14.46±0.433 ^c
25	71.17±0.63 ^d	5.77±0.29 ^d	23.46±0.29 ^c	18.83±0.777 ^d

Values are expressed in mean ± standard error (n=10). Values with different letters are significantly different (p<0.05).

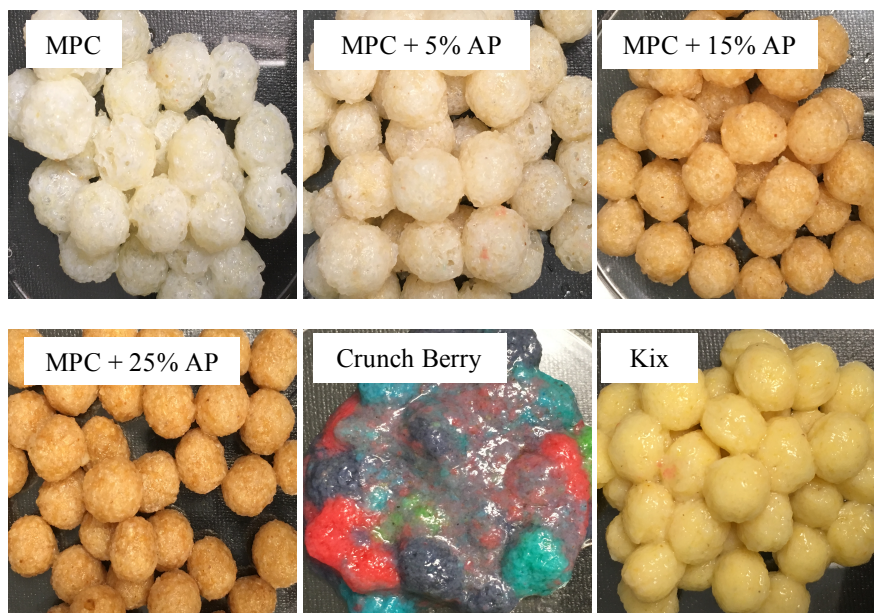


Fig 6. MPC extrudates with AP and commercial products after submersion in DI water for 5 minutes

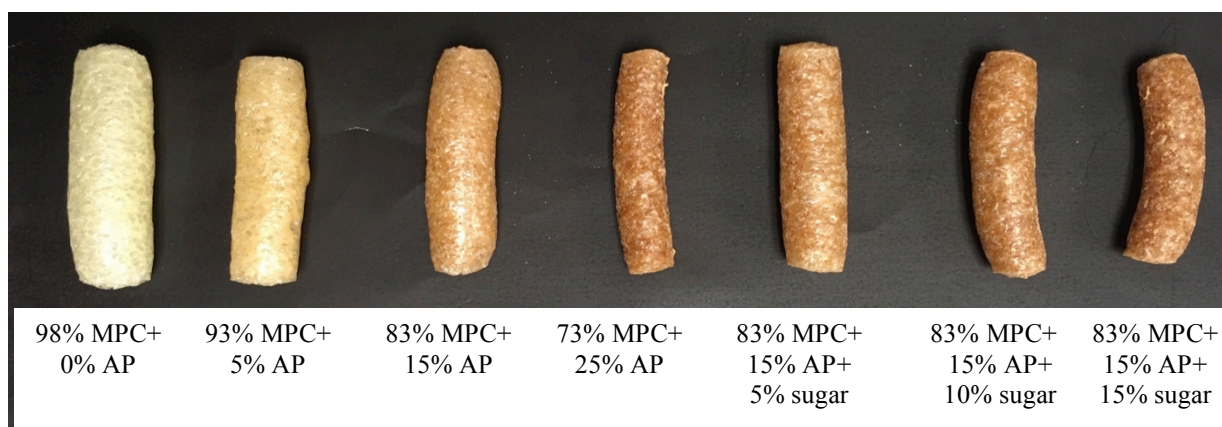


Fig 7. Photograph of MPC extrudates with added AP and sugar

Mechanical properties of MPC extrudates with added AP

Results from mechanical properties analysis showed a curvilinear response during the initial stages of the stress-strain curves of the extrudates (Fig 8). No elastic region was present in the stress-strain plots and the modulus varied continuously from the start of compression to the point of fracture. The changes in modulus describe the anisotropic

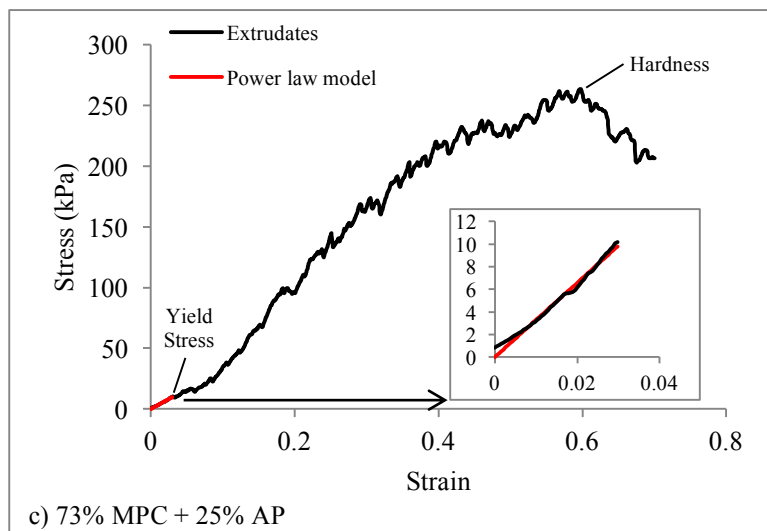
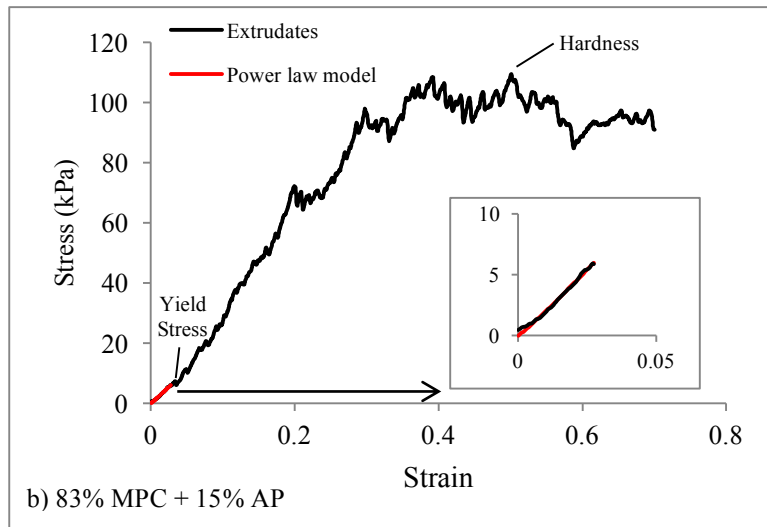
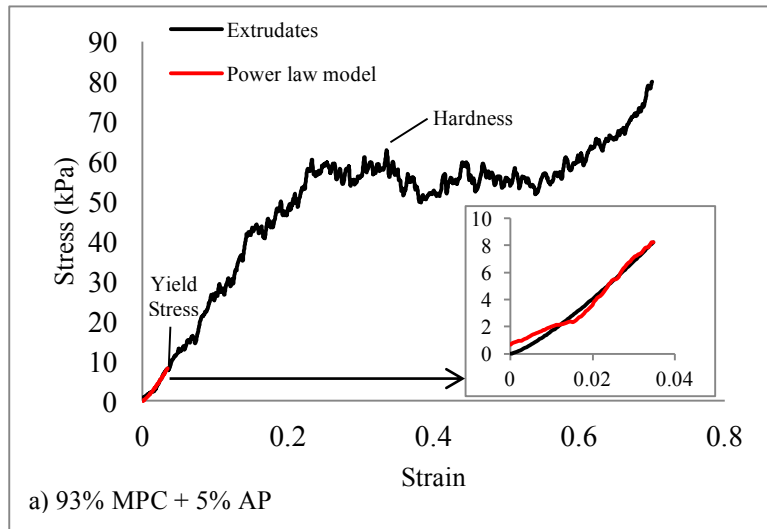
porosity of the cells. This type of cellular anisotropy has been related to high dough-elasticity during extrusion (Karkle et al., 2012; Pai et al., 2009). A power law-like equation (Eq 8) was used to quantify the curvilinear behavior in the stress-strain curve of the extrudates at a water activity of 0.2. Equation 8 has two parameters, M and n , which describe the effect of anisotropic porosity on the mechanical properties of the MPC extrudates. The values of n decreased when the AP concentration was increased (Table 9) and in turn the extrudates porosity was decreased. The curvilinear behavior disappeared completely ($n=1$) when the added AP concentration reached 25% (Fig 8c) and the porosity was at its lowest. When the value n equals 1, parameter M becomes the modulus of elasticity of the sample. Similar to the result from a previous study (Karkle et al., 2012), the anisotropy disappeared due to added AP since the fiber in AP aligned at the extrudates cell walls and reduced the elastic recovery which lead to isotropic cell growth during expansion.

Added AP also affected the textural qualities of MPC extrudates. Adding 15% AP significantly decreased the crispness and significantly increased the hardness and yield stress of the MPC extrudates (Fig 9). As reported in other studies (Karkle et al., 2012; O'Shea et al., 2013; Onwulata et al., 2001; Robin et al., 2011), the textural properties of extruded products are correlated with their porosities. Porosity is also negatively correlated with the thickness of the cell wall (Karkle et al., 2012). Higher AP concentrations in the MPC extrudates caused lower porosities that correspond with fewer air cells within the extrudates and therefore thicker cell walls. Thicker walls

gave more resistance during compression, and thus increased the hardness and yield stress.

Effects of AP on the crispness were also evident, in which added AP decreased the normalized number of peaks in the stress-strain curve of the extrudates (Fig 9). The fiber alignment in the cell walls of the extrudates caused the cell walls to have abrupt fractures which self-propagated, instead of a greater number of smaller, successive fractures extended over the period of compression.

Higher moisture contents had negative effects on the mechanical properties of puffed products as reported by Heidenreich et al. (2004) and Mazumder et al. (2007). However, the texture of the MPC extrudates with low AP concentrations did not show significant hardening and crispness loss due to moisture even when the water activity was increased to 0.6 (Fig 9). Increasing the AP concentration to 15% caused more pronounced texture degradations due to increased moisture. Loss of crispness was observed as the result of plasticization. When AP was added, the T_g of the extrudates decreased, thus the extrudates lost their brittle foam characteristics and plastic deformation replaced the small, successive fractures. Furthermore, hardness (Fig 9a) and yield stress (Fig 9c) of MPC extrudates with 25% AP increased significantly due to moisture. Swelling of fiber molecules due to moisture caused the extrudates to behave like a compact solid.



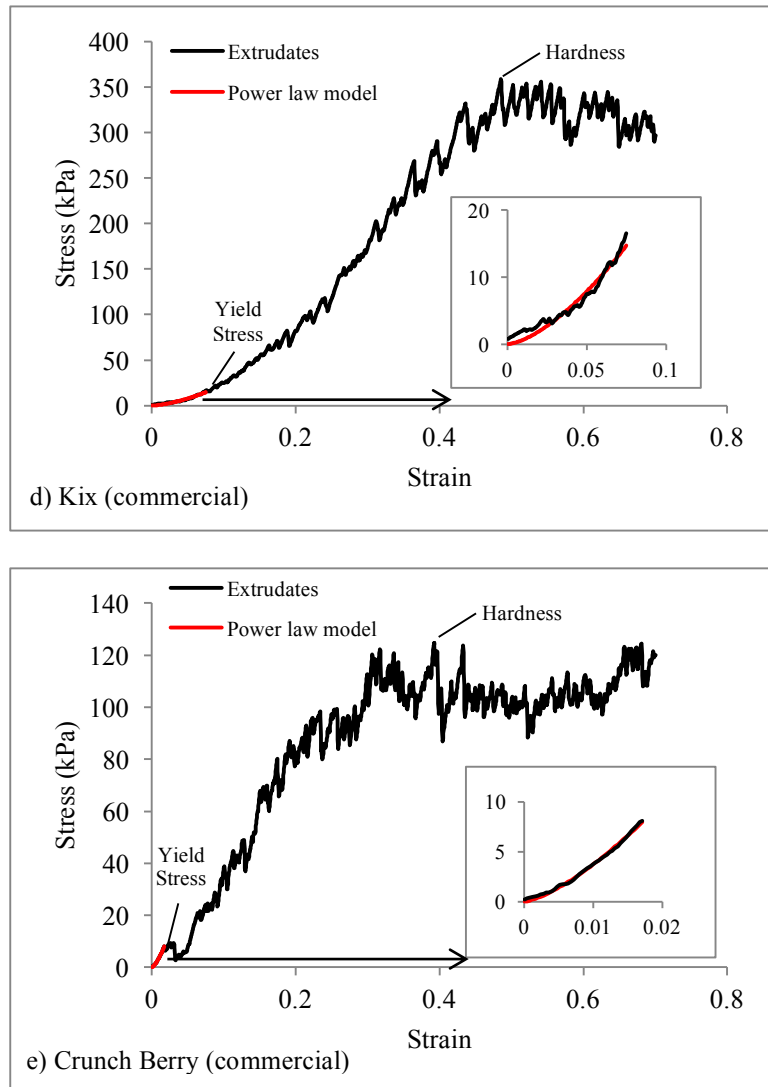
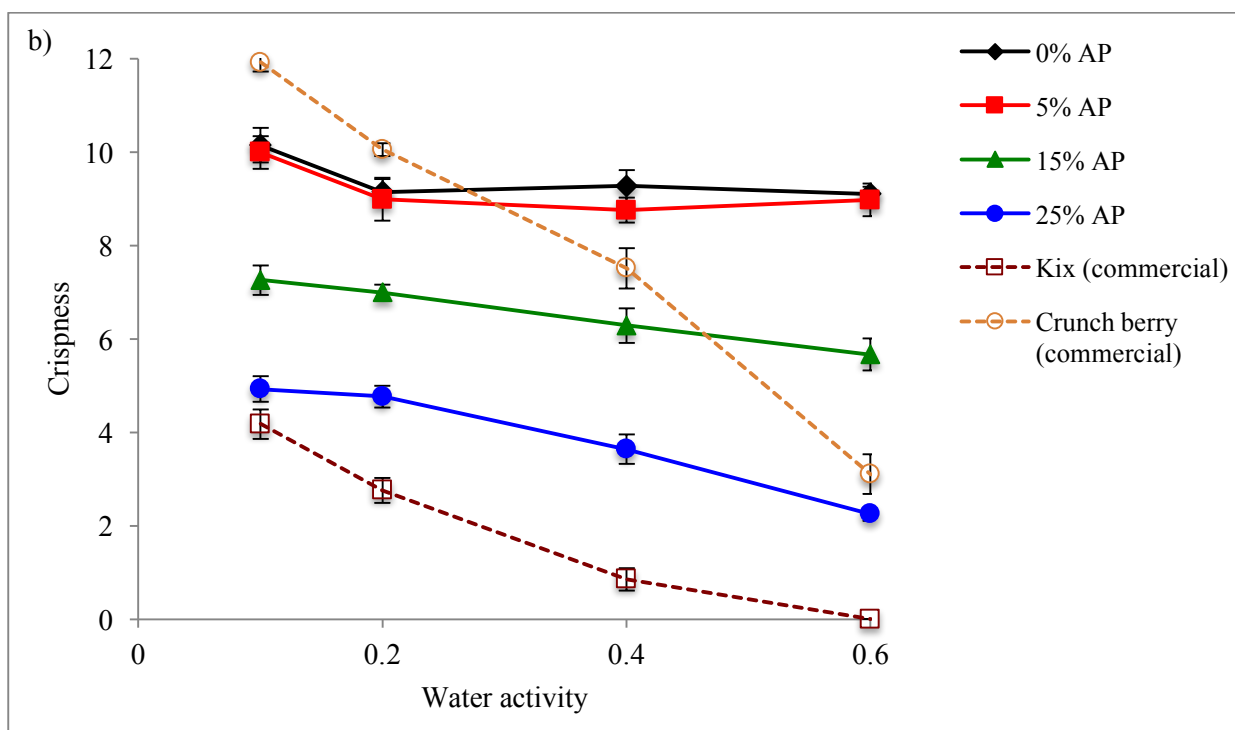
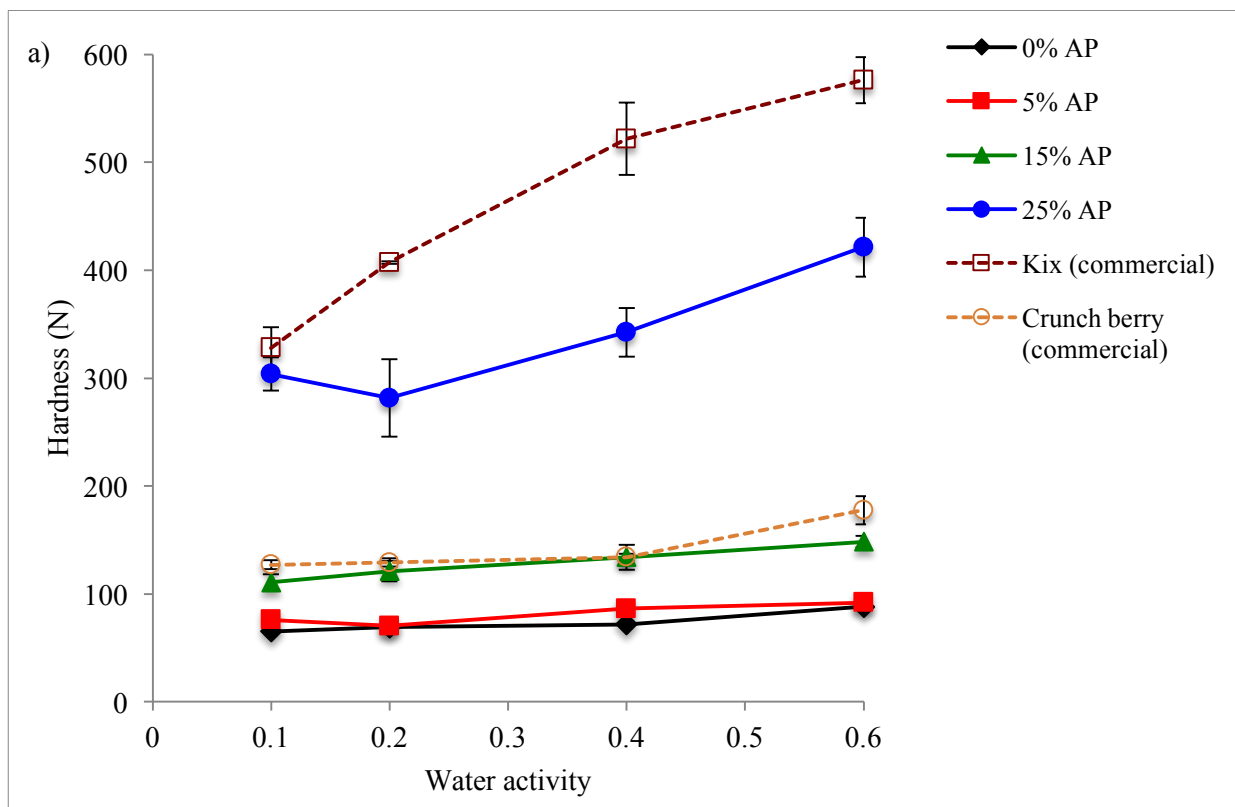


Fig 8. Stress-Strain curve of MPC extrudates with added AP and commercial products: a) 5% AP b) 15% AP c) 25% AP d) Kix (commercial) e) Crunch Berry (commercial)

Table 9. Power law parameters from stress-strain curve fitting

Sample	M	n
MPC	871.30	1.27
MPC + 5% AP	581.10	1.26
MPC + 15% AP	286.06	1.07
MPC + 25% AP	323.49	1.00
<i>Commercial products:</i>		
Kix	786.50	1.54
Crunch Berry	2438.79	1.41



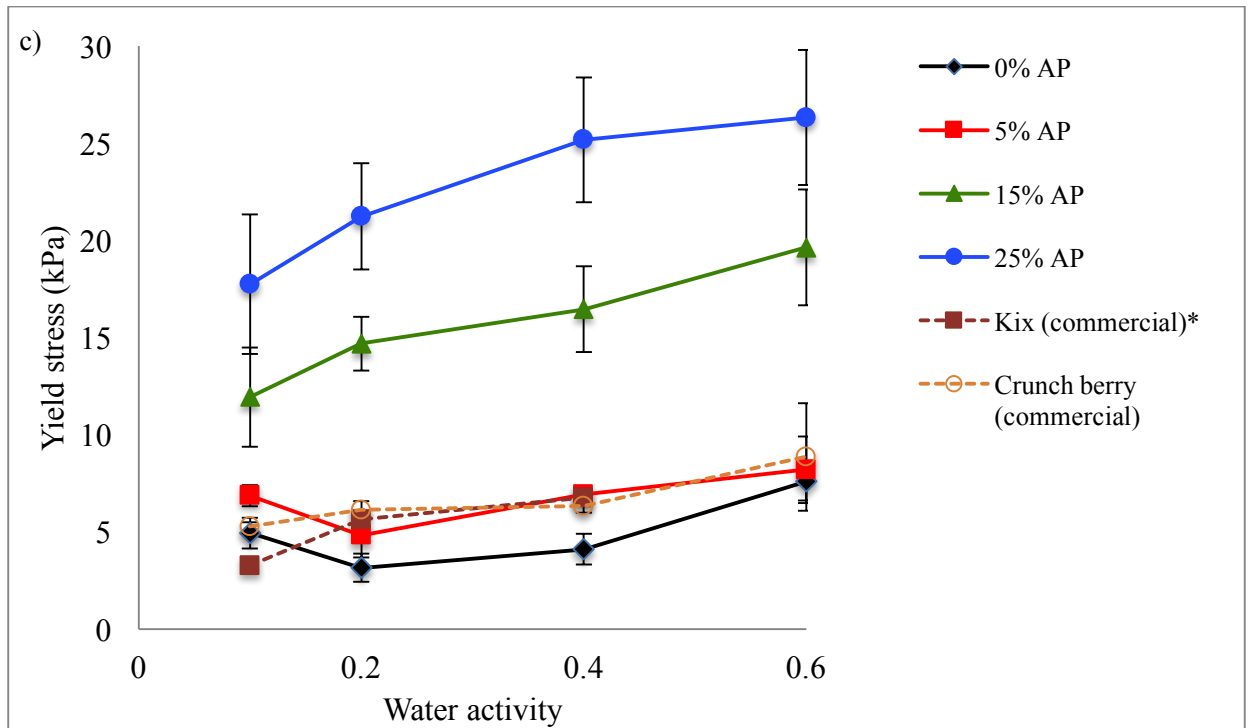


Fig 9. The effects of water activity on: (a) hardness, (b) crispness and (c) yield stress of the MPC extrudates with AP and the commercial products. Points are mean value (n=6) with standard error bar.

* : yield stress of Kix at a_w 0.6 are not detected

Effects of added sugar

Three concentrations of sugar solutions were used to replace water during SCFX. The results of the present study were in agreement with previous studies (Barrett et al., 1995; Pitts et al., 2014), where high concentration (15%) of added sugar affected the RER, density, porosity (Table 10), hardness and crispness (Table 11) of the MPC extrudates. At high concentration, sucrose eventually inhibited the ability of the dough to expand due to the increased viscosity and decreased elasticity as indicated by the increasing SME. On the other hand, low sugar concentrations (5-10%) had no adverse effect on the expansion, density, porosity and texture of the extrudates (Table 10,

Table 11) since the sugar acted as a plasticizer in the dough and lowered the energy needed during the expansion. However, added sugar had a pronounced effect on the color of MPC extrudates. Even 5% added sugar was enough to significantly promote Maillard browning in MPC extrudates (Table 12).

Table 10. Physical properties of MPC-AP extrudates with added sugar

Added sugar (wt.%)	RER	Piece density (g/cm ³)	Porosity
0	8.68±1.68 ^a	0.17±0.03 ^a	0.76±0.05 ^a
5	8.16±0.45 ^a	0.24±0.01 ^b	0.67±0.01 ^a
10	8.89±0.58 ^a	0.17±0.01 ^a	0.78±0.01 ^a
15	6.28±0.56 ^b	0.36±0.06 ^c	0.52±0.09 ^b

Values are expressed in mean±standard error. Different letters indicates significant difference (p<0.05)

Table 11. Texture of MPC-AP extrudates with added sugar

Added sugar (wt.%)	Hardness (N)	Crispness	M ¹	N ¹
0	121.15±23.05 ^a	6.70±0.40 ^a	286.06	1.07
5	132.46±13.32 ^{ab}	5.73±0.58 ^{ab}	1056.491	1.31
10	180.13±9.55 ^b	5.38±1.38 ^b	3071.337	1.57
15	335.32±35.39 ^c	3.52±0.71 ^c	821.011	1.04

Values are expressed in mean±standard error. Different letters indicates significant difference (p<0.05)

¹Power law parameter of the initial part of stress-strain curve

Table 12. Color of MPC-AP extrudates with added sugar

Added sugar (wt.%)	Color			
	L	a	b	ΔE
0	75.01±0.91 ^a	3.44±0.09 ^a	18.47±0.52 ^a	15.33±0.67 ^a
5	70.20±0.41 ^b	4.40±0.07 ^b	17.78±0.09 ^a	19.60±0.43 ^b
10	69.96±0.59 ^b	4.44±0.13 ^b	17.67±0.22 ^a	19.81±0.72 ^b
15	70.15±0.34 ^b	4.62±0.09 ^b	18.53±0.11 ^a	19.88±0.66 ^b

Values are expressed in mean±standard error. Different letters indicates significant difference (p<0.05)

Effect of SCFX on the total phenolic content of extrudates

As expected, higher added AP concentration increased the total phenolic content of both the mixed formulation powders and the extrudates. The measured total phenolic contents in MPC-AP extrudates were also increased after SCFX (Table 13). SCFX

minimized the thermal degradation of the phenolic compounds during extrusion (Leyva-Corral et al., 2016) because SC-CO₂ allowed the puffing process to be done at a low temperature. The shear force inside the barrel also broke some of the AP's cell structure and increased the extractability of the phenolic compounds (Brennan et al., 2011).

Table 13. Total phenolic content of MPC extrudate

AP Concentration (wt.%)	Total phenolic content before extrusion (mg GAE/g)	Total phenolic content after extrusion (mg GAE/g)	%increase
5	0.132±0.001 ^a	0.191±0.003 ^b	45%
15	0.262±0.002 ^b	0.406±0.031 ^c	55%
25	0.373±0.007 ^c	0.653±0.012 ^d	75%
MPC powder	0.052±0.001	-	-
AP powder	1.708±0.009	-	-

Values are expressed in mean±standard error. Different letters indicates significant difference (p<0.05)

2.4. Conclusions

SCFX was effectively utilized to extrude MPC and AP into well-expanded, low-density products with minimal browning and with textural qualities comparable to commercial products. These extrudates were shelf-stable during 9 months of storage without any significant textural degradation. As expected, high added AP concentration ($\geq 15\%$) not only reduced the radial expansion ratio, porosity, crispness, and water diffusion coefficient but also increased the density, hardness and yield stress of the MPC extrudates. Furthermore, added sugar can be incorporated into the extrudates at low concentrations ($\leq 10\%$) without any significant effect on the physical qualities. SCFX retained the total phenolic contents in apple pomace and even increased their availability due to cell breaking. Understanding the properties of MPC-based extrudates with added AP and sugar allows the manufacturing of

nutritionally enhanced puffed snacks or breakfast cereals containing pomace from other fruits and related ingredients.

2.5. Suggestion for Future Research

MPC extrudates have good textural properties and they are stable during storage without any hardening and loss of crispness. Thus, MPC extrudates can be exploited as crisps in the development of high-protein nutrition bar with crunchy texture and prolonged storage time.

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